InterLab Study Lab Notebook

Danny + Erica + Jack
Jack
Erica
Kosuke
Danny + Kirsten + Jack
Kirsten + Jack

08.18.15

- Reviewed requirements and goals for InterLab Study (Danny + Erica + Jack)
  - Three biological replicates are required for each device
  - Will be using flow cytometry to measure GFP
- Transformed NEB 5-alpha cells with BBa_J23101 (BB1), BBa_J23106 (BB2), BBa_J23117 (BB3), and BBa_I13504 (Pt1) (Danny + Erica + Jack)
  - Plated BB1, BB2, and BB3 on LB chloramphenicol plates (as they are in pSB1C3 backbones), and plated Pt1 on an LB ampicillin plate (as it is in a pSB1A2 backbone)
  - Let grow overnight in 37°C incubator

08.19.15 (Jack)

- Picked two colonies from each transformation plate from previous day and grew in liquid cultures overnight
  - BB1, BB2, and BB3 colonies were grown in LB chloramphenicol broth
  - Pt1 colonies were grown in LB ampicillin broth

08.20.15 (Erica)

- Mini-prepped BB1, BB2, BB3, and Pt1 plasmids

08.22.15 (Erica)

- Performed double digests of the six parts
  - BB1, BB2, and BB3 were digested with SpeI and PstI
  - Pt1 was digested with XbaI and PstI
  - Digestions used 250 ng of plasmid DNA, 4 µL of CutSmart buffer, and 1 µL of each restriction enzyme. DI MilliQ water was used to bring the reaction volume up to 40 µL.
- Performed T4 ligation of BB1, BB2, and BB3 with Pt1

- Transformed NEB 5-alpha competent cells with the three ligation products, along with a positive control and negative control
    - Positive control is BBa_I20270, as suggested in Interlab Protocol form
    - Negative control is BBa_R0040, as suggested in Interlab Protocol form
    - Grew overnight in 37ºC incubator

08.23.15

- Moved plates from 37ºC incubator to 4ºC fridge (Kosuke)

08.24.15

- Visualized transformation plates from previous day (Danny + Kirsten + Jack)
- Picked three GFP-expressing colonies from each experimental plate, and grew in 4 mL LB chloramphenicol cultures in 37ºC incubator (Kirsten + Jack)
    - J23117 + GFP didn't have colonies that appeared fluorescent, so 8 colonies were picked in hopes that at least 3 contained the promoter + GFP
- Picked 3 colonies from each control plate, and grew in 4 mL LB chloramphenicol cultures in 37ºC incubator (Kirsten + Jack)

08.25.15

- Moved liquid cultures to 4ºC fridge (Erica)

08.27.15

- Mini-prepped 2 mL of each liquid culture (Danny + Erica)
- Sent out experimental and positive control plasmids for sequencing (Danny + Erica + Thai)
- Measured fluorescence with incorrect parameters (ex. 501 em. 511 cut-off 495) (Erica)

08.28.15

- Analyzed sequencing data (Danny + Erica)
- Measured fluorescence of control and experimental liquid cultures (Erica)
    - Each measurement was taken three times (i.e. three technical replicates were performed) at more optimal settings (ex. 485 em. 528 cut-off 495)
    - Discussed with Griffin how we don't currently have the means to measure absolute fluorescence